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(54) Title: UNCOUPLING PROTEIN HOMOLOGUE: UCP 3

(57) Abstract

The present invention relates to the cloned genes which code for uncoupling proteins controlling thermogenesis in human skeletal muscle and heart. A further aspect of the present invention relates to the use of the said genes for correcting dysfunctions of thermogenesis in human skeletal muscle and heart. The present invention makes it possible to exploit novel therapeutic (or preventive) methods for disorders such as obesity or cachexia. As a result of the identification and isolation of the genes coding for UCP3_L and UCP3_S, it is, in effect, possible to develop medicaments which act on the basis of a correction, by gene therapy or by antisense oligonucleotides relating to the sequence of the gene in question or to one of its fragments, of a lack or an excess of UCP3.

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UNCOUPLING PROTEIN HOMOLOGUE: UCP 3

The present invention relates to a cloned gene which codes for an uncoupling protein (UCP3₁) which controls thermogenesis in human skeletal muscle and heart.

The present invention also relates to a cloned gene which codes for an uncoupling protein (UCP3_S), also controlling thermogenesis in human skeletal muscle and heart.

A further aspect of the present invention relates to the use of the said cloned genes for correcting dysfunctions of thermogenesis in human skeletal muscle and heart.

A dysfunction of thermogenesis can induce disorders such as obesity or cachexia.

Obesity is characterized by an excess of adipose mass which can represent more than 30% of the bodyweight. The incidence of this disturbance of energy balance is constantly increasing in industrialized countries. Preventing the development of obesity, or treating it, would enable the complications associated with this pathology, namely cardiovascular diseases, hypertension and type II diabetes, to be avoided.

In some special cases, the control of thermogenesis could also prove useful. In man, weight loss following a slimming diet induces a saving of energy by the body. On resumption of normal feeding, the energy expenditure remains reduced until the body has recovered the adipose mass and the lean mass which it has lost previously. This decrease in energy expenditure often leads to an excess weight regain. A similar problem is encountered in endurance athletes as soon as they stop training. In effect, in a trained person, the energy expenditure is decreased relative to that of a sedentary person. This energy saving is responsible for a substantial weight gain (most especially of fat) after chronic physical activity has been stopped.

Cachexia is a metabolic situation in which the energy expenditure exceeds the food intake. Its main causes are underfeeding (e.g. anorexia nervosa), cancer, infectious diseases including AIDS and a state of shock. The decrease in adipose and muscle masses can threaten the individual's survival.

The energy expenditure is increased in the mitochondria by uncoupling of the oxidative phosphorylations. The oxidations induce the exit of protons (H⁺) from the mitochondrion, creating a proton gradient which, as it dissipates, permits the synthesis of

ATP. Uncoupling can be induced by chemical compounds such as 2,4-dinitrophenol (DNP) and by other acidic aromatic compounds. These substances carry H⁺ from the outside to the inside of the inner mitochondrial membrane. In the presence of these uncoupling agents, the oxidation of NADH takes place normally, but ATP is not formed by the mitochondrial ATP synthetase since the proton gradient is dissipated.

Brown adipose tissue (BAT), which is very rich in mitochondria, is specialized in this process of thermogenesis. The inner membrane of its mitochondria contains a large amount of an uncoupling protein (UCP) which enables protons to return from the outside to the inside of the mitochondrion. In essence, the uncoupling protein produces heat by short-circuiting the battery of mitochondrial protons. In spite of its tissue specificity, UCP is a member of the mitochondrial carrier family, which includes the ATP/ADP, phosphate and 2-oxoglutarate/malate carriers, in particular. In contrast to the ATP/ADP carrier, which is a constitutive carrier, UCP is subjected to substantial regulatory processes (M. Klingenberg, *J. Bioenerg. Biomembr.* **25**, 447 (1993)). Its activity is decreased by purine di- or triphosphate nucleotides and increased by fatty acids (J. Nedergaard, B. Cannon, in *New Comprehensive Biochemistry (Bioenergetics)* L. Ernster, Ed (Elsevier Science, Stockholm, 1992), vol. 23, 385).

The uncoupling of oxidative phosphorylation is very useful from a biological standpoint; it is a means for BAT to generate heat in order to maintain a physiological temperature in the newborn offspring of some animals (including man) and in the mammals of the cold regions.

The human UCP gene is localized at 4q31; it consists of six exons and codes for a protein of 307 amino acids with no targeting presequence signal. Like other mitochondrial carriers, UCP is inserted into the mitochondrial membrane by six hydrophobic α -helical domains, each encoded by a portion of the six exons (L.P. Kozak et al., *J. Biol. Chem.* **263**, 12274 (1988)). Its polypeptide chain consists of three related sequences in tandem of approximately 100 amino acids, each encoded by two exons and corresponding to two transmembrane domains (F. Bouillaud et al., *Biochem. Biophys. Res. Commun.* **157**, 783 (1988)). Topological studies suggest that the amino- and carboxy-terminal ends of UCP are oriented towards the cytosol side of the inner mitochondrial membrane (B. Miroux et al., *EMBO J.* **12**, 3739 (1993)). Furthermore, J. Nedergaard et al. (*New Comprehensive Biochemistry (Bioenergetics)* L. Ernster, Ed. (Elsevier Science, Stockholm, 1992), vol. 23, 385) and D. Ricquier et al. (*FASEB J.* **5**, 2237 (1991)) have shown that the expression of

UCP is increased at transcriptional level by noradrenaline. This effect is mediated by stimulation of the three subtypes of β -adrenoceptors (β_1 , β_2 and β_3) and of the α_1 -adrenoceptor.

However, M.E.J. Lean et al. (*Brown Adipose Tissue* P. Trayhurn, D.G. Nicholls, Eds, (Edward Arnold, London, 1986), 339) have shown that, in man, the BAT, expressing UCP, present in newborn infants decreases considerably in adults. Hence, under physiological conditions, BAT cannot play an important part in non-shivering thermogenesis in man.

On the other hand, L. Simonsen et al. (*Int. J. Obes.* 17, S47 (1993)) have determined that, in man, skeletal muscle contributes to the extent of approximately 40% to adrenaline-induced body thermogenesis. Studies performed on rats (P.L. Thurlby et al., *Can. J. Physiol. Pharmacol.* 64, 1111 (1986); I. Nagase et al., *J. Clin. Invest.* 97, 2898 (1996)) suggest that adrenaline-induced thermogenesis in human skeletal muscle (L. Simonsen et al., *Int. J. Obes.* 17, S47 (1993)) could be mediated by a muscle UCP.

In the search for homologues of UCP in human skeletal muscle, we have screened a library of human skeletal muscle cDNA, and three clones (UCP2, UCP3_L and UCP3_S) have been isolated. Whereas UCP2 mRNA has been found in all human tissues studied, as has also been described by Fleury et al. (*Nature Genet.* 15, 269 (1997)), UCP3 has proved to be very specific to human skeletal muscle. This new member of the UCP family, with a strong specificity of expression in skeletal muscle, is involved in the control of oxidative phosphorylations in man.

The characterization of the genes coding for UCP3_L and UCP3_S in terms of nucleotides and of amino acids is described here for the first time.

One aspect of the present invention is hence a DNA fragment, characterized in that it contains the nucleotide sequence depicted in SEQ ID NO 3, the said DNA fragment coding for an uncoupling protein (UCP3_L) which is characterized by the amino acid sequence depicted in SEQ ID NO 4, or in that it contains a homologous sequence coding for the same amino acid sequence. Preferably, the chosen DNA fragment originates from human skeletal muscle.

Another aspect of the present invention is a DNA fragment, characterized in that it contains the nucleotide sequence depicted in SEQ ID NO 5, the said DNA fragment coding for an uncoupling protein (UCP3_S) possessing the amino acid sequence depicted in

SEQ ID NO 6, or in that it contains a homologous sequence coding for the same amino acid sequence. Preferably, the chosen DNA fragment originates from human skeletal muscle.

One subject of the present invention is an uncoupling protein, characterized in that it comprises the amino acid sequence depicted in SEQ ID NO 4.

Another subject of the present invention is an uncoupling protein, characterized in that it comprises the amino acid sequence depicted in SEQ ID NO 6.

Furthermore, the present invention sets out to provide a recombinant DNA fragment which contains one of the nucleotide sequences depicted in SEQ ID NO 3 or NO 5, or one of their homologous sequences.

Another subject of the present invention provides a DNA molecule, characterized in that it comprises a cloning vector into which one of the said DNA sequences is inserted. Preferably, this cloning vector is a plasmid or a phagemid.

Another subject of the present invention is a recombinant DNA molecule, characterized in that it consists of the nucleotide sequence depicted in SEQ ID NO 3, inserted into the vector pBluescript SK⁺. This recombinant DNA molecule is deposited with the ATCC, Rockville, Md. 20852 USA (ATCC Designation NO 97999 - date of deposition: April 25, 1997).

Another subject of the present invention is a recombinant DNA molecule, characterized in that it consists of the nucleotide sequence depicted in SEQ ID NO 5, inserted into the vector pBluescript SK⁺. This recombinant DNA molecule is deposited with the ATCC, Rockville, Md. 20852 USA (ATCC Designation NO 209000 - date of deposition: April 25, 1997).

Another subject of the present invention is a microorganism selected from bacteria, yeasts and mammalian cells, characterized in that it contains one of the said recombinant DNA molecules. Preferably, the chosen microorganism is an XL1-Blue MRF' bacterium (*E. coli*).

Another aspect of the present invention relates to a pharmaceutical formulation for correcting a lack of UCP3 by gene therapy, which comprises the gene described in SEQ ID NO 3 or NO 5 and a suitable pharmaceutical vehicle. The said gene is preferably contained in a vector chosen from adenoviruses, retroviruses, adeno-associated viruses, herpesvirus, liposomes or DNA plasmids.

Construction of the adenoviral vector and production of the recombinant adenovirus may be accomplished using standard techniques (Graham et al., *Method in Molecular Biology*, 1991, Vol. 7, chap. 11, Murray, E.J., Ed., The Humana Press, Inc.,

Clifton, NJ; Zabner et al., *Cell*, **75**, 207-216 (1993); Crystal, et al., *Nature Genetics*, **8**, 42-51 (1994)).

Construction of the retroviral vector and production of the recombinant retrovirus may be accomplished using standard techniques (Miller, A.D. et al., *Meth. Enzymol.*, **217**, 581-599 (1993); Blaese et al., *Human Gene Therapy*, **1**, 331-362, (1990)).

Construction of the adeno-associated viral vector and production of the recombinant adeno-associated virus may be accomplished using standard techniques (Muzyczka, *Curr. Top. Microbiol. Immunol.*, **158**, 97-129 (1992)).

Construction of the herpesviral vector and production of the recombinant herpesvirus may be accomplished using standard techniques (Glorioso et al., *Semin. Virol.*, **3**, 265-276 (1992)).

Production of the liposome containing the UCP3 gene may be accomplished using standard techniques (Kirby et al., *Biotechnology*, **2**, 979-984 (1984); Felgner et al., *Proc Natl Acad Sci USA*, **84**, 7413-7417 (1987); Gregoriadis et al., *J. Drug Targeting*, **3**, 467-475 (1996)).

Construction of the DNA plasmid and production of the recombinant DNA plasmid may be accomplished using standard techniques (Lee et al., *Cancer Res.*, **54**, 3325-3328 (1994)). The functionality of the recombinant retroviral vector, the recombinant adeno-associated viral vector, the recombinant herpesviral vector, the UCP3 gene encapsidated in the liposome, and the recombinant DNA plasmid may, in each case, be evaluated by the expression of UCP3 in transfected cells and by the presence of the UCP3 protein, using the northern blot technique (Sambrook, et al., *Molecular Cloning*, 1989, Nolan, C., Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.) and the western blot technique (Sambrook, et al., *Molecular Cloning*, 1989, Nolan, C., Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.), respectively.

In addition, a subject of the present invention is a medicament for correcting an excess of UCP3, which comprises as active principle antisense oligonucleotides relating to fragments of the sequences of UCP3_L and of UCP3_S.

The present invention thus makes it possible to exploit novel therapeutic (or preventive) methods for disorders of the abovementioned type. As a result of the identification and isolation of the genes coding for UCP3_L and UCP3_S, it is, in effect, possible to develop medicaments which act on the basis of a correction, by gene therapy or

by antisense oligonucleotides relating to the sequence of the gene in question or to one of its fragments, of a lack or an excess of UCP3.

Gene therapy is indicated in the case of a dysfunction of UCP3 due to one or more mutations of the UCP3 gene. In this case, there is transfer of a normal gene into the UCP3-deficient cells using an appropriate vector. Preferably, the vector is chosen from adenoviruses, retroviruses, adeno-associated viruses, herpesvirus, liposomes or plasmids. In this case the UCP3 gene is under the control of an exogenous promoter. The administration of antisense oligonucleotides is specifically indicated for cases where the UCP3 level and/or the activity of the UCP3 might be excessive.

By means of the present invention, it will, in addition, be possible to develop medicaments which act on the modification of the activity and/or on the level of expression of endogenous UCP3.

Intervention in respect of the modification of endogenous UCP3 activity could be especially indicated for inducing a loss of bodyweight (loss of adipose mass and maintenance of the lean mass) in all types of obesity by promoting the dissipation of energy. It was shown that the level of UCP3 mRNA in skeletal muscle is lower in fa/fa obese rats than in lean Fa/? rats (O. Boss et al., *J. Biol. Chem.* **273**, 5 (1998)). The same intervention could also be applied for preventing an excessive weight regain following a restrictive food diet or after ceasing a physical training programme. It was shown that food restriction induces a decrease in the UCP3 gene expression in skeletal muscle (O. Boss et al., *J. Biol. Chem.* **273**, 5 (1998)), and this expression remains very low for a prolonged period of time during refeeding (D.W. Gong et al., *J. Biol. Chem.* **272**, 24129 (1997)). It was also reported that endurance exercise training induces a decrease in the UCP3 gene expression in skeletal muscle (O. Boss et al., *FASEB J* **12**, 335 (1998)). The same intervention could be applied for preventing and treating type II diabetes by improving the sensitivity to insulin, and for preventing hypertension. The same intervention could also be applied for increasing muscle mass in states of cachexia. It could also be applied for the treatment of insufficiencies or disturbances of cardiac rhythm due to a dysfunction of UCP3, or for the treatment of neuromuscular diseases due to a dysfunction of UCP3. The UCP3 activators or inhibitors could be administered enterally or parenterally.

Modification of the level of expression of endogenous UCP3 would also be indicated for the abovementioned cases; intervention could take place using activators or inhibitors of UCP3 expression. These substances could be administered enterally or

parenterally. Possible candidates for modulating the expression would be activators or inhibitors of transcription factors specific to UCP3, or hormones. It was shown that thyroid hormones (T3), glucocorticoids (dexamethasone), β -adrenergic receptor agonists (Ro 16-8714), leptin, and fatty acids induce an increase in the UCP3 gene expression in skeletal muscle (D.W. Gong et al., *J. Biol. Chem.* **272**, 24129 (1997), S. Larkin et al., *Biochem. Biophys. Res. Com.* **240**, 222 (1997), O. Boss et al., Unpublished observations (1997), I. Cusin et al., *Diabetes*, (in press, 1998), D.S. Weigle et al., *Diabetes* **47**, 298 (1998)). On the other hand hypothyroidism was shown to be associated with a lower level of UCP3 mRNA in skeletal muscle (D.W. Gong et al., *J. Biol. Chem.* **272**, 24129 (1997)).

The construction of cells containing the UCP3_L and/or UCP3_S gene may be accomplished using standard techniques (*Methods in Molecular Biology*, 1991, Vol. 7, chap. 2-5, Murray, E.J., Ed., The Humana Press, Inc., Clifton, NJ; Sambrook, et al., *Molecular Cloning*, 1989, Nolan, C., Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.). The expression of the UCP3 gene in the transfected cells may be evaluated by the northern blot technique and western blot technique. Cultured C₂C₁₂ mouse myoblasts have been transfected with the human UCP3_L cDNA, and the expression of UCP3 has been evaluated by the northern blot technique (O. Boss et al., *J. Biol. Chem.* **273**, 5 (1998)). The functionality of the UCP3 protein may be evaluated by measuring the respiration on isolated cells expressing the UCP3 protein or mitochondrial fractions (*Methods in Enzymology*, Vol. 10, chap. 14, 86-94 (1967)) containing the UCP3 protein. The measurements of oxygen consumption are performed by polarography (*Methods in Enzymology*, Vol. 10, chap. 7, 41-48 (1967)) using a Clark electrode. It is also possible to evaluate the activity of the UCP3 protein using cytofluorometric methods for measuring the mitochondrial membrane potential (*Methods in Enzymology*, Vol. 260, chap. 29, 406-417, chap. 31, 428-447 (1985)). Fluorescent compounds are used to analyse the changes in mitochondrial membrane potential. With the help of a cytofluorometric method the UCP3 has been shown to decrease the mitochondrial membrane potential of C₂C₁₂ mouse myoblasts that have been transfected with the human UCP3_L cDNA (O. Boss et al., *J. Biol. Chem.* **273**, 5 (1998)). A screening of medicaments capable of modifying UCP3 activity could be accomplished by measuring the oxygen consumption and/or the mitochondrial membrane potential on isolated cells and/or mitochondria containing the UCP3 protein. Medicaments that modify the activity of the UCP3 protein could then be tested in vivo on animal models displaying an excess or otherwise a defect of UCP3 activity.

Antibodies, or their derivatives, against UCP3 or UCP3 fragments could be developed in order to enable the UCP3 level to be measured in biological samples for diagnostic purposes. These antibodies could serve to target substances in proximity to UCP3 with a view to modifying UCP3 activity.

In addition, transgenic animals expressing an excess of normal or modified UCP3, and animals with an invalidation of the UCP3 (knock-out), could be created in order to permit an evaluation of the biological role of UCP3 and/or the effects of a change in activity and/or in level and/or in structure of the UCP3 on the animal's biology. The transgenic animals could also be used to screen or test substances that modify UCP3 expression and/or activity.

Other advantages of the present invention will become manifest on reading the description which follows.

Brief description of the figures as disclosed in O. Boss et al., *FEBS Lett.* **408**, 39 (1997) which are hereby incorporated by reference into the present specification:

Figure 1: Alignment of the amino acid sequences of human UCP3_L, UCP3_S, UCP2 and UCP, obtained using the ClustalW Multiple Sequence Alignment programme (K.C. Worley, Human Genome Center, Baylor College of Medicine). The sequences are presented according to the one-letter code. The spaces introduced into the sequences to optimize the alignment are illustrated by a dash. The potential transmembrane α -helices are underlined and numbered using Roman numbers (I-VI). The potential purine nucleotide binding domain (PNBD) is doubly underlined. The 3 signature domains of the mitochondrial energy transfer proteins are boxed. The 14 amino acids which are fully conserved in the mitochondrial carriers are indicated with asterisks.

Figure 2: Expression of UCP2 and UCP3 in human and rat tissues. (A) Autoradiogram of a northern blot of poly(A) RNA of various human tissues hybridized with a ³²P-labelled human UCP2 probe consisting of whole UCP2 cDNA. The molecular size markers are indicated in kb. Inset: Expression of UCP2 in total RNAs. Brown adipose tissue (BAT); white adipose tissue (WAT). (B) Autoradiogram of a northern blot of poly(A) RNA of various human tissues hybridized with a ³²P-labelled human UCP3 probe consisting of whole UCP3 cDNA. Inset: Expression of UCP3 in total RNAs. (C) Autoradiogram of a

northern blot of total RNAs of various rat tissues. The ^{32}P -labelled rat atypical UCP cDNA probe hybridizes with the three rat UCPs, UCP (1.4 kb), UCP2 (1.7 kb) and UCP3 (2.5 and 2.8 kb).

Preparation of a rat atypical UCP cDNA probe and screening of the human skeletal muscle cDNA library (O. Boss et al., *FEBS Lett.* **408**, 39 (1997))

Total RNAs of rat tibialis anterior muscle and interscapular BAT were purified by the method described by Chomczynski et al. (*Anal. Biochem.* **162**, 156 (1987)). 1-2 μg of each RNA were reverse transcribed using oligo(dT)₁₅ primers and Moloney murine leukaemia virus (M-MLV) reverse transcriptase (Gibco BRL, New York, NY). Briefly, the RNA was mixed with 1.5 μl (150 ng) of oligo(dT)₁₅ in a total volume of 17.5 μl . The mixture was heated to 70°C for 10 min and cooled on ice. After a brief centrifugation, the RNA was reverse transcribed for one hour at 41°C (total volume 25 μl). The aliquots of first strand cDNA were stored at -20°C until use. The polymerase chain reaction was performed in a Perkin Elmer DNA Thermal Cycler 480 (Perkin Elmer, Lausanne, Switzerland). The first strand cDNA (2 μl) was amplified in a total volume of 50 μl in the presence of oligonucleotide primers corresponding, on rat UCP, to domains conserved between the species:

positions 279-298: CTGGACACCGCCAAAGTCCG (UCPRF)

positions 1021-1044: AGCACACAAACATGATGACGTTCC (UCPRR)

on GenBank Accession M11814. A unique fragment of similar size was obtained in the BAT and in the tibialis anterior muscle. The sequence of the BAT PCR product was identical to that of rat UCP, whereas the sequence of the tibialis anterior muscle PCR product was 60% identical to that of rat UCP. This fragment, called rat atypical UCP cDNA, was radioactively labelled with the nucleotide [α - ^{32}P]dCTP and used as probe to screen a human skeletal muscle cDNA library (Stratagene, #936215, La Jolla, CA).

Approximately one million phages of the human skeletal muscle cDNA library (Human Muscle cDNA Library Lambda ZAP® II Vector, #936215, Stratagene) were screened using the abovementioned rat atypical UCP cDNA probe according to the manufacturer's instructions (8th March 1994). The screening led to the isolation of ten positive clones. Among these, three different categories were purified using a kit for plasmids (Qiagen, Santa Clarita, CA) according to the manufacturer's instructions, and sequenced with an ABI373A automatic sequencer according to the standard protocols using the

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oligonucleotide primers M13-20 and T3 and others specific to the gene, until the sequence was determined on both strands. The predicted peptide sequences of these three clones, which contain 309, 312 and 275 amino acids, are illustrated in Fig. 1 (O. Boss et al., *FEBS Lett.* **408**, 39 (1997)). The protein of 309 amino acids is UCP2 (GenBank Accession U82819), which was also isolated by Fleury et al., (*Nature Genet.* **15**, 269 (1997)). The other two proteins have their first 275 amino acids in common, suggesting that they are isoforms. It was shown that these isoforms arise from alternative splicing of the UCP3 RNA (G. Solanes et al., *J. Biol. Chem.* **272**, 25433 (1997)). They have a 56 % and 73 % identity of amino acids with human UCP and UCP2, respectively, and are hence considered to be new proteins, called UCP3 long and short forms, UCP3_L and UCP3_S, respectively (GenBank Accessions U84763 and U82818). Like the other mitochondrial carriers, UCP2 and UCP3_L contain six potential transmembrane domains (Fig. 1). The potential purine nucleotide binding domain extends from nucleotides 276 to 298 in UCP2 and from 279 to 301 in UCP3_L. UCP3_S does not have the sixth potential transmembrane region, or the purine nucleotide binding domain involved in the control of the uncoupling activity of UCP (F. Bouillaud et al., *EMBO J.* **13**, 1990 (1994)).

The binding of guanosine diphosphate (GDP) to UCP induces a conformational change causing an inhibition of the permeability of H⁺ and Cl⁻ ions (J. Nedergaard et al., in *New Comprehensive Biochemistry (Bioenergetics)* L. Ernster, Ed. (Elsevier Science, Stockholm, 1992), vol. 23, 385). Thus, UCP3_S appears to exert its biological activity without control by GDP. As illustrated in Fig. 1, other structural features of the mitochondrial carriers are observed in UCP2, as well as in UCP3_L and UCP3_S: the three signature domains of the mitochondrial energy transfer proteins (A. Bairoch, *Nucleic Acids Res.* **21**, 3097 (1993)), which can be identified at the downstream border of the first, third and fifth potential transmembrane domains (O. Boss et al., *FEBS Lett.* **408**, 39 (1997)), and the fourteen conserved residues of the mitochondrial carriers (F. Palmieri, *FEBS Lett.* **346**, 48 (1994)).

UCP2 and UCP3 have the strongest homology (with respect to amino acids and nucleotides) with UCP, compared to the other members of the mitochondrial carrier family (O. Boss et al., *FEBS Lett.* **408**, 39 (1997)). In fact, their identity with UCP is 55 % and 56 %, respectively, whereas that with the most homologous mitochondrial protein, the 2-oxoglutarate/malate carrier, is 32 %. UCP2 and UCP3 should hence belong to the UCP family. In fact, Fleury et al., (*Nature Genet.* **15**, 269 (1997)) and Gimeno et al. (*Diabetes* **46**, 900 (1997))

have shown that UCP2 has mitochondrial uncoupling properties when it is expressed in yeast, whereas Gong et al. (*J. Biol. Chem.* **272**, 24129 (1997)) and Boss et al. (*J. Biol. Chem.* **273**, 5 (1998)) have shown that UCP3 has mitochondrial uncoupling properties when it is expressed in yeast or in C₂C₁₂ mouse myoblasts, respectively.

Comparison of the distribution of UCP2 and UCP3 in human and rat tissues. (O. Boss et al., *FEBS Lett.* **408**, 39 (1997))

The tissue distribution of UCP3 was compared with that of UCP2 by northern blotting. For this purpose, the following materials were made available:

- Samples of Human Tissues

The poly(A) RNA membrane of numerous human tissues (#7760-1) is obtained from Clontech Laboratories Inc. (Palo Alto, CA). Fragments of abdominal white adipose tissue (10 to 40 g) or of abdominal skeletal muscle (800 mg) were obtained during intra-abdominal surgical operations. Pieces of perirenal brown adipose tissue weighing approximately 1.5 g were obtained during renal surgical operations on children (average age: 3 months). The project was approved by the Ethical Committee of the Department of Surgery, Faculty of Medicine, University of Geneva.

- Samples of Rat Tissues

Seven-week-old male Sprague-Dawley rats fed ad libitum with a standard laboratory feed were kept in individual cages with a day-night cycle of 12 h. They were sacrificed by cervical dislocation. All the experiments were performed in accordance with the instructions of our establishment.

Northern Analysis

The total RNAs (10-20 µg) were subjected to electrophoresis in a 1.2% agarose gel containing formaldehyde, as described by Lehrach et al., (*Biochemistry* **16**, 4743 (1977)), and transferred onto a nylon membrane (Electran Nylon Blotting Membrane, BDH Laboratory Supplies, Poole, United Kingdom) with a vacuum apparatus (Stratagene, La Jolla, CA). The probes were labelled using random primers (Megaprime DNA Labelling System, Amersham, Bucks, United Kingdom) with [α -³²P]dCTP (3000 Ci/mmol) (Amersham, Bucks, United Kingdom) at a specific activity of approximately 1×10^9 dpm/µg DNA. The RNA membranes were hybridized for 2 h at 65°C in QuikHyb (Stratagene, La Jolla, CA), and then washed in a 2×SSC (1×SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0)/0.1 % SDS solution at 50°C for twice 5 minutes and in 0.1×SSC/0.1 % SDS at 50°C for 5 minutes. The

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membranes were exposed to Hyperfilm ECL films (Amersham, Bucks, United Kingdom) at - 80°C with intensifying screens. The standard RNAs used are the Kb RNA Ladder from Gibco BRL (New York, NY).

As illustrated in Fig. 2A, a UCP2 signal 1.7 kb in size was detected in all the tissues studied. UCP2 is expressed at the highest level in BAT > white adipose tissue > skeletal muscle. In contrast, the expression of UCP3 (signal at 2.3 kb) is limited to the skeletal muscle and the heart. In the latter tissue, UCP3 is 10 times less strongly expressed than in skeletal muscle (Fig. 2B). Direct comparison of the northern blots hybridized with the UCP2 and UCP3 probes showed that UCP3 is much more strongly expressed in skeletal muscle than UCP2. Probes specific for the long form or the short form of UCP3 showed that both forms give the same signal at 2.3 kb, and quantification of the intensity of the signal showed that both forms are expressed at a similar level in human skeletal muscle (O. Boss et al., *FEBS Lett.* **408**, 39 (1997)).

The tissue distribution of UCP2 mRNA and UCP3 mRNA was also studied in rats using the rat atypical UCP cDNA probe, which hybridizes with the three species of rat UCP, UCP, UCP2 and UCP3 (O. Boss et al., *FEBS Lett.* **408**, 39 (1997)). As illustrated in Fig. 2C, UCP2 is expressed in all the tissues studied: heart > BAT > white adipose tissue > skeletal muscle. A major difference from human UCP2 is its high level of expression in heart. UCP3 is expressed at the highest level in BAT, at a high level in the tensor fascia latae (fast-twitch, glycolytic), tibialis anterior (fast-twitch, oxidative-glycolytic) and gastrocnemius (mixed) muscles and at a lower level in the soleus muscle (slow-twitch, oxidative). This suggests that UCP3 is more strongly expressed in the glycolytic than in the oxidative skeletal muscles. In rats, UCP3 was also detected, though at a much lower level, in the heart and kidney, and occasionally in the white adipose tissue.

It can be seen in Fig. 2C that the UCP3 signal is a doublet whereas that of UCP2 is unique. The size of the messengers, compared with an RNA ladder, are 1.4 and 1.8 kb for UCP, 1.7 kb for UCP2 and 2.5 and 2.8 kb for UCP3.

The results thus show that the tissue distribution of UCP2 and UCP3 in man is very different from that of UCP: the expression of UCP2 is ubiquitous and that of UCP3 is highly specific to skeletal muscle.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: NOVARTIS AG
- (B) STREET: SCHWARZWALDALLEE 215
- (C) CITY: Basel
- (E) COUNTRY: Switzerland
- (F) POSTAL CODE (ZIP): 4058
- (G) TELEPHONE: +41 61 324 11 11
- (H) TELEFAX: + 41 61 322 75 32

(ii) TITLE OF INVENTION: Isolation and Characterization of a Gene

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Oligonucleotide primer DNA (UCPRF)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CTGGACACCG CCAAAGTCGG

20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: Oligonucleotide primer DNA (UCPRR)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AGCACACAAA CATGATGACG TTCC

24

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1231 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

(B) CLONE: UCP3L

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TCCTGGGATG GAGCCCTAGG GAGCCCTGT GCTGCCCTG CCGTGGCAGG ACTCACAGCC	60
CCACCGCTGC ACTGAAGCCC AGGGCTGTGG AGCAGCCTCT CTCCCTGGAC CTCCCTCTGG	120
CCCTAAAGGG ACTGGGCAGA GCCTTCCAGG ACTATGGTG GACTGAAGCC TTCAGACGTG	180
CCTCCACCA TGGCTGTGAA GTTCCCTGGGG CCAGGCACAG CAGCCTGTTT TGCTGACCTC	240
GTTACCTTTC CACTGGACAC AGCCAAGGTC CGCCTGCAGA TCCAGGGGA GAACCAGGCG	300
GTCCAGACGG CCCGGCTCGT GCAGTACCGT GGC GTGCTGG GCACCATCCT GACCATGGTG	360
CGGACTGAGG CTCCCTGCAG CCCCTACAAT GGGCTGGTGG CGGGCCTGCA GCGCCAGATG	420
AGCTTCGCCT CCATCCGCAT CGGCCTCTAT GACTCCGTCA AGCAGGTGTA CACCCCCAAA	480

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GGCGCGGACA ACTCCAGCCT CACTACCCGG ATTTGGCCG GCTGCACCCAC AGGAGCCATG	540
GCGGTGACCT GTGCCAGCC CACAGATGTG GTGAAGGTCC GATTCAGGC CAGCATAACAC	600
CTCGGGCCAT CCAGGAGCGA CAGAAAATAC AGCGGGACTA TGGACGCCCTA CAGAACCATC	660
GCCAGGGAGG AAGGAGTCAG GGGCCTGTGG AAAGGAACCT TGCCCAACAT CATGAGGAAT	720
GCTATCGTCA ACTGTGCTGA GGTGGTGACC TACGACATCC TCAAGGAGAA GCTGCTGGAC	780
TACCACCTGC TCACTGACAA CTTCCCCCTGC CACTTTGTCT CTGCCCTTG AGCCGGCTTC	840
TGTGCCACAG TGGTGGCCTC CCCGGTGGAC GTGGTGAAGA CCCGGTATAT GAACTCACCT	900
CCAGGCCAGT ACITCAGCCC CCTCGACTGT ATGATAAAAGA TGGTGGCCCA GGAGGGCCCC	960
ACAGCCTCT TACAAGGGATT TACACCCCTCC TTTTTGCGTT TGGGATCCTG GAACTGGTG	1020
ATGTTCGTAA CCTATGAGCA GCTGAAACGG GCCCTGATGA AAGTCCAGAT GTTACGGAA	1080
TCACCGTTT GAACAAGACA AGAAGGCCAC TGGTAGCTAA CGTGTCCGAA ACCAGTTAAG	1140
AATGGAAGAA AACGGTGCAT CCACGCACAC ATGGACACAG ACCCACACAT GTTTACAGAA	1200
CTGTTGTTA CTGTTGCTG ATTCAAGAAA C	1231

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 312 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vii) IMMEDIATE SOURCE:

(B) CLONE: UCP3L

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Val Gly Leu Lys Pro Ser Asp Val Pro Pro Thr Met Ala Val Lys			
1	5	10	15

Phe Leu Gly Ala Gly Thr Ala Ala Cys Phe Ala Asp Leu Val Thr Phe

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20	25	30	
Pro Leu Asp Thr Ala Lys Val Arg	Leu Gln Ile Gln Gly Glu Asn Gln		
35	40	45	
Ala Val Gln Thr Ala Arg	Leu Val Gln Tyr Arg Gly Val Leu Gly Thr		
50	55	60	
Ile Leu Thr Met Val Arg Thr Glu Gly	Pro Cys Ser Pro Tyr Asn Gly		
65	70	75	80
Leu Val Ala Gly Leu Gln Arg Gln Met Ser	Phe Ala Ser Ile Arg Ile		
85	90	95	
Gly Leu Tyr Asp Ser Val Lys Gln Val Tyr	Thr Pro Lys Gly Ala Asp		
100	105	110	
Asn Ser Ser Leu Thr Thr Arg	Ile Leu Ala Gly Cys Thr Thr Gly Ala		
115	120	125	
Met Ala Val Thr Cys Ala Gln Pro Thr Asp	Val Val Lys Val Arg Phe		
130	135	140	
Gln Ala Ser Ile His Leu Gly Pro Ser Arg	Ser Asp Arg Lys Tyr Ser		
145	150	155	160
Gly Thr Met Asp Ala Tyr Arg Thr Ile Ala Arg	Glu Glu Gly Val Arg		
165	170	175	
Gly Leu Trp Lys Gly Thr Leu Pro Asn Ile Met Arg	Asn Ala Ile Val		
180	185	190	
Asn Cys Ala Glu Val Val Thr Tyr Asp	Ile Leu Lys Glu Lys Leu Leu		
195	200	205	
Asp Tyr His Leu Leu Thr Asp Asn Phe Pro Cys	His Phe Val Ser Ala		
210	215	220	
Phe Gly Ala Gly Phe Cys Ala Thr Val Val	Ala Ser Pro Val Asp Val		
225	230	235	240
Val Lys Thr Arg Tyr Met Asn Ser Pro Pro	Gly Gln Tyr Phe Ser Pro		
245	250	255	
Leu Asp Cys Met Ile Lys Met Val Ala Gln	Glu Gly Pro Thr Ala Phe		
260	265	270	
Tyr Lys Gly Phe Thr Pro Ser Phe Leu Arg	Leu Gly Ser Trp Asn Val		
275	280	285	
Val Met Phe Val Thr Tyr Glu Gln Leu Lys	Arg Ala Leu Met Lys Val		
290	295	300	
Gln Met Leu Arg Glu Ser Pro Phe			
305	310		

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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1132 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

- (B) CLONE: UCP3S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TCCTGGGATG GAGCCCTAGG GAGCCCTGT GCTGCCCTTG CCGTGGCAGG ACTCACAGCC	60
CCACCGCTGC ACTGAAGCCC AGGGCTGTGG AGCAGCCTCT CTCCTTGGAC CTCCCTCTCGG	120
CCCTAAAGGG ACTGGGGAGA GCCTTCCAGG ACTATGGTIG GACTGAAGCC TTCAGACGTG	180
CCTCCACCA TGGCTGTGAA GTTCCCTGGGG GCAGGCACAG CAGCCTGTTT TGCTGACCTC	240
GTTACCTTTC CACTGGACAC AGCCAAGGTG CGCCTGCAGA TCCAGGGGGA GAACCAGGGG	300
GTCCAGACGG CCCGGCTCGT GCAGTACCGT GGCGTGCTGG GCACCATCCT GACCATGGTG	360
CGGACTGAGG GTCCCTGCAG CCCCTACAAT GGGCTGGTGG CCGGCCTGCA GCGCCAGATG	420
AGCTTCGCCT CCATCCGCAT CGGCCTCTAT GACTCCGTCA AGCAGGTGTA CACCCCCAAA	480
GGCGCGGACA ACTCCAGCCT CACTACCCGG ATTTTGGCCG GCTGCACCAC AGGAGCCATG	540
GCGGTGACCT GTGCCAGCC CACAGATGTG GTGAAGGTCC GATTCAGGC CAGCATAACAC	600
CTCGGGCCAT CCAGGAGCGA CAGAAAATAC AGCGGGACTA TGGACGCCTA CAGAACCATC	660
GCCAGGGAGG AAGGAGTCAG GGGCCTGTGG AAAGGAACCT TGCCCAACAT CATGAGGAAT	720
GCTATCGTCA ACTGTGCTGA GGTGGTGACC TACGACATCC TCAAGGAGAA GCTGCTGGAC	780
TACCACTGTC TCACTGACAA CTTCCCTGC CACTTGTCT CTGCCCTTGG AGCCGGCTTC	840
TGTGCCACAG TGGTGGCCTC CCCGGTGGAC GTGGTGAAGA CCCGGTATAT GAACTCACCT	900
CCAGGCCAGT ACTTCAGCCC CCTCGACTGT ATGATAAAGA TGGTGGCCCA GGAGGGCCCC	960

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ACAGCCTCT ACAAGGGGTG AGCCTCCCTCC TGCCTCCAGC ACTCCCTCCC AGAGAACAGG	1020
GGCTTCTTTC TTTTCGAATG TGGCTACCGT GGGTCAACCT GGGATGTAGC GGTGAAGAGT	1080
ACAGATGTAA ATGCCACAAA GAAGAAGTTT AAAAAACCAT GCAAAAAAAAA AA	1132

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 275 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) IMMEDIATE SOURCE:

(B) CLONE: UCP3S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Val Gly Leu Lys Pro Ser Asp Val Pro Pro Thr Met Ala Val Lys			
1	5	10	15

Phe Leu Gly Ala Gly Thr Ala Ala Cys Phe Ala Asp Leu Val Thr Phe		
20	25	30

Pro Leu Asp Thr Ala Lys Val Arg Leu Gln Ile Gln Glu Asn Gln		
35	40	45

Ala Val Gln Thr Ala Arg Leu Val Gln Tyr Arg Gly Val Leu Gly Thr		
50	55	60

Ile Leu Thr Met Val Arg Thr Glu Gly Pro Cys Ser Pro Tyr Asn Gly			
65	70	75	80

Leu Val Ala Gly Leu Gln Arg Gln Met Ser Phe Ala Ser Ile Arg Ile		
85	90	95

Gly Leu Tyr Asp Ser Val Lys Gln Val Tyr Thr Pro Lys Gly Ala Asp		
100	105	110

Asn Ser Ser Leu Thr Thr Arg Ile Leu Ala Gly Cys Thr Thr Gly Ala		
115	120	125

Met Ala Val Thr Cys Ala Gln Pro Thr Asp Val Val Lys Val Arg Phe

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130	135	140	
Gln Ala Ser Ile His Leu Gly Pro Ser Arg Ser Asp Arg Lys Tyr Ser			
145	150	155	160
Gly Thr Met Asp Ala Tyr Arg Thr Ile Ala Arg Glu Glu Gly Val Arg			
165	170	175	
Gly Leu Trp Lys Gly Thr Leu Pro Asn Ile Met Arg Asn Ala Ile Val			
180	185	190	
Asn Cys Ala Glu Val Val Thr Tyr Asp Ile Leu Lys Glu Lys Leu Leu			
195	200	205	
Asp Tyr His Leu Leu Thr Asp Asn Phe Pro Cys His Phe Val Ser Ala			
210	215	220	
Phe Gly Ala Gly Phe Cys Ala Thr Val Val Ala Ser Pro Val Asp Val			
225	230	235	240
Val Lys Thr Arg Tyr Met Asn Ser Pro Pro Gly Gln Tyr Phe Ser Pro			
245	250	255	
Leu Asp Cys Met Ile Lys Met Val Ala Gln Glu Gly Pro Thr Ala Phe			
260	265	270	
Tyr Lys Gly			
275			

CLAIMS

1. DNA fragment, characterized in that it contains the nucleotide sequence depicted in SEQ ID NO 3, the said DNA fragment coding for an uncoupling protein (UCP3_L) which is characterized by the amino acid sequence depicted in SEQ ID NO 4, or in that it contains a homologous sequence coding for the same amino acid sequence.
2. DNA fragment according to Claim 1, characterized in that it originates from human skeletal muscle.
3. DNA fragment, characterized in that it contains the nucleotide sequence depicted in SEQ ID NO 5, the said DNA fragment coding for an uncoupling protein (UCP3_S) possessing the amino acid sequence depicted in SEQ ID NO 6, or in that it contains a homologous sequence coding for the same amino acid sequence.
4. DNA fragment according to Claim 3, characterized in that it originates from human skeletal muscle.
5. Uncoupling protein, characterized in that it comprises the amino acid sequence depicted in SEQ ID NO 4.
6. Uncoupling protein, characterized in that it comprises the amino acid sequence depicted in SEQ ID NO 6.
7. Recombinant DNA fragment, characterized in that it comprises a DNA sequence according to Claims 1, 2, 3 or 4, or one of their homologous sequences.
8. DNA molecule, characterized in that it comprises a cloning vector into which a DNA sequence according to one of Claims 1 to 4 or 7 is inserted.
9. DNA molecule according to Claim 8, characterized in that the cloning vector is a plasmid or a phage.
10. Recombinant DNA molecule according to Claim 9, characterized in that it consists of the nucleotide sequence depicted in SEQ ID NO 3, inserted into the vector pBluescript SK⁺.
11. Recombinant DNA molecule according to Claim 10, deposited with the ATCC (NO 97999).
12. Recombinant DNA molecule according to Claim 9, characterized in that it consists of the nucleotide sequence depicted in SEQ ID NO 5, inserted into the vector pBluescript SK⁺.
13. Recombinant DNA molecule according to Claim 12, deposited with the ATCC (NO 209000).

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14. Microorganism selected from bacteria, yeasts and mammalian cells, characterized in that it contains a recombinant DNA molecule as claimed in Claims 7 to 13.
15. Microorganism according to Claim 14, characterized in that it is an XL1-Blue MRF' bacterium (*E. coli*).
16. Pharmaceutical formulation for correcting a lack of UCP3 (UCP3_L, UCP3_S) by gene therapy, which comprises the gene described in SEQ ID NO 3 or NO 5 and a suitable pharmaceutical vehicle.
17. Pharmaceutical formulation according to Claim 16, characterized in that the said gene is contained in a vector chosen from adenoviruses, retroviruses, adeno-associated viruses, herpesvirus, liposomes or DNA plasmids.
18. Pharmaceutical formulation for correcting an excess of UCP3 (UCP3_L, UCP3_S), characterized in that it comprises as active principle, antisense oligonucleotides relating to fragments of the sequences of UCP3_L or of UCP3_S.

INTERNATIONAL SEARCH REPORT

national Application No

PCT/EP 98/02645

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C12N1/21 A61K48/00 C12N15/86
A61K31/70

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>BOSS O ET AL: "Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression." FEBS LETT, MAY 12 1997, 408 (1) P39-42, XP002067895 NETHERLANDS cited in the application see the whole document</p> <p>-----</p> <p>VIDAL-PUIG A ET AL: "UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue." BIOCHEM BIOPHYS RES COMMUN, JUN 9 1997, 235 (1) P79-82, XP002075964 UNITED STATES see the whole document</p> <p>-----</p> <p>-----</p>	1-7
P, X		1-7

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

31 August 1998

Date of mailing of the international search report

14/09/1998

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Esben, J

INTERNATIONAL SEARCH REPORT

I. International Application No

PCT/EP 98/02645

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	DA-WEI GONG ET AL: "Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 39, 26 September 1997, pages 24129-24132, XP002075965 MD US cited in the application see the whole document ---	1-9, 14
Y	FLEURY C et AL: 'Human uncoupling protein-2 (UCP2) mRNA, nuclear gene encoding mitochondrial protein, complete CDS' EMHUM Database entry HSU76367 Accession number U76367; 06-MAR-1997 XP002075999 see sequence ---	1-9, 14
Y	HILLIER L ET AL: 'Homo sapiens cDNA clone 628529 5' similar to TR:G412267 UNCOUPLING PROTEIN' EMEST Database entry Hsaa98452 Accession number AA192136; 21-01-1997 XP002076000 see sequence ---	1-9, 14
Y	MARRA M ET AL: 'Mus musculus cDNA clone 570531 5' similar to SW:UCP_RABIT P14271 MITOCHONDRIAL BRWON FAT UNCOUPLING PROTEIN' EMEST Database entry Mmaa8362 Accession number AA108362; 06-NOV-1996 XP002076001 see sequence ---	1-9, 14
Y	WO 96 05861 A (MILLENIUM PHARM INC) 29 February 1996 see claims 1,3; figures 16,17 -----	1-9, 14

INTERNATIONAL SEARCH REPORT

Information on patent family members

1 National Application No

PCT/EP 98/02645

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9605861	A 29-02-1996	US 5741666 A	US 3497295 A	21-04-1998
		AU 5702902 A		14-03-1996
				30-12-1997